

**WHAT IS CLAIMED IS:**

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1. A method for the identification of interacting protein, said method comprising:  
a) subjecting an extract to protein-affinity chromatography on multiple columns, said columns having a protein ligand coupled to the column matrix in varying concentrations, and eluting bound components of said extract from said columns;  
b) separating said components to isolate an interacting protein;  
c) analyzing the interacting protein by mass spectrometry to identify the interacting protein.
2. The method of claim 1, wherein said columns are micro-columns.
3. The method of claim 1, wherein said separation is a gel-separation.
4. The method of claim 3, wherein said gel-separation is a polyacrylamide gel electrophoresis.
5. ~~The method of claim 4, wherein said polyacrylamide gel does not contain SDS.~~
6. The method of claim 1, wherein said protein ligand is covalently bound to the matrix.
7. The method of claim 1, wherein said mass spectrometry is MALDI-TOF mass spectrometry.
8. The method of claim 1, wherein the bound components of the extract are eluted with a protein denaturant
9. A method for the identification of an interacting protein, said method comprising:  
a) subjecting a cellular extract or extracellular fluid to protein-affinity chromatography on multiple columns, said columns having a protein ligand coupled to the column matrix in varying concentrations, and eluting bound components of said extract from said columns;

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- b) gel-separating said components to isolate an interacting protein; wherein the interacting protein is observed to vary in amount in direct relation to the concentration of coupled protein ligand;
  - c) digestion of said interacting protein to give corresponding peptides
  - d) analyzing said peptides by MALDI-TOF mass spectrometry or post source decay to determine the peptide masses, and
  - e) correlative database searching with said peptide or peptide fragment masses, whereby the interacting protein is identified

10 10. The method of claim 9, wherein said columns are micro-columns.

11. The method of claim 9, wherein said gel-separation is a polyacrylamide gel electrophoresis.

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15 12. ~~The method of claim 11, wherein said polyacrylamide gel does not contain SDS.~~

13. The method of claim 9, wherein said protein ligand is covalently bound to the matrix.

14. The method of claim 9, wherein the identities of the interacting protein partners are entered into a relational database.

20 15. The method of claim 9, wherein the bound components of the extract are eluted with a protein denaturant.

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